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PATENT #24
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Theodore W. RANDOLPH *et al.*

Serial No.: 09/350,327

Filed: July 9, 1999

For: HIGH PRESSURE REFOLDING OF PROTEIN AGGREGATES AND INCLUSION BODIES

Group Art Unit: 1651

Examiner: H. Guttman

Atty. Dkt. No.: UTEC:003/SLH

Considered
6 June 02

DECLARATION OF C. RUSSELL MIDDAUGH UNDER 37 C.F.R. §1.132

Hon. Commissioner for Patents
Washington, D.C. 20231

I, C. Russell Middaugh, declare that:

1. I am a U.S. citizen residing at 849 East 1000 Rd., Lawrence, KS.

2. I currently am the Aya and Takeru Higuchi Distinguished Professor of Pharmaceutical Chemistry at the University of Kansas School of Pharmacy, where I have worked since 1997. I am also the Associate Editor for Journal of Pharmaceutical Sciences and on the Editorial Board

for Journal of Biological Chemistry. Prior to taking this position, I was a Senior Scientist and Director at Merck & Co. A copy of my *curriculum vitae* is attached.

3. I have over 25 years of research experience in the areas of protein folding, stability and aggregation. In particular, over 100 of my more than 150 published papers are in this area. I also worked for many years as the Director of Protein Formulation for Merck & Co., where I worked on a regular basis on related protein folding problems.

4. I have reviewed the Office Action for the above-captioned application dated July 21, 2001, as well as the Zong *et al.* (1995) reference cited therein. I have also reviewed the pending claims for the present invention, as well as the specification therein. After review these materials, it is my conclusion that Zong *et al.* (1995) does not teach the use of aggregates in a high pressure refolding process as is claimed in the present application.

5. In my expert opinion, the paper of Zong *et al.* (1995) in no way teaches how to refold aggregating proteins by employing high pressure conditions. In fact, it is quite clear form the text of the paper that aggregated forms of the protein are removed **prior** to the refolding steps. This is clearly stated on page 12421, col. 1. Furthermore, the material employed by Zong *et al.* (1995) in their actual refolding experiments does not appear to be aggregated based on the spectral properties of the starting material. Circular dichroism, fluorescence and absorption spectroscopy all manifest certain distinctive spectral artifacts (*e.g.*, absorption flattening, differential light scattering, *etc.*) in the presence of aggregated protein that are quite recognizable and are not present in the data of Figures 5, 6 and 7. In contrast, the claims of the present

application are very clear in being drawn to the use of protein aggregates, and subsequent refolding under high pressure to eliminate aggregation.

6. In light of these observations, I would disagree with the examiner's conclusion that Zong *et al.* (1995) teaches the use of protein aggregates.

7. I hereby declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

30 April 2002
Date

C. Russell Middaugh
C. Russell Middaugh

CURRICULUM VITAE

I. Personal

A. Name: Charles Russell Middaugh, Ph.D.

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II. Education

School	Date	Major	Degree
University of California Santa Cruz	1973	Chemistry & Biology	B.S.
Cornell University	1978	Biochemistry	Ph.D.

III. Employment History

Dates	Position	Location
1997-Present	Aya and Takeru Higuchi Distinguished Professor of Pharmaceutical Chemistry	University of Kansas, Department of Pharmaceutical Chemistry
1989-1997	Senior Scientist, Merck & Co.	Pharmaceutical Research, Merck Vaccine Division, and Department of Human Genetics

Responsibilities included basic structure/function studies of proteins and small molecules, formulation design for peptides, proteins and vaccines, and the development of delivery systems for gene therapy.

1986 - 1987	Visiting Scientist	Massachusetts Institute of Technology
1984 - 1989	Associate Professor	University of Wyoming
1979 - 1984	Assistant Professor	University of Wyoming
1977 - 1978	NIH Postdoctoral Fellow	University of Minnesota
1998	Kenneth E. Avis Distinguished Visiting Professor	University of Tennessee

V. Representative Examples of Recent Academic and Professional Activities

Regular Member: NIH Physiological Chemistry Study Section 1987-1991 NIH Reviewers Reserve (NRR) 1991-1995.

Keynote Speaker, Third Internat'l. Conference on Biotechnology, Seoul, Korea, 1990.

Merck Centennial Lecturer, Univ. of Kansas, 1991.

Merck Centennial Lecturer, Purdue Univ., 1991.

Co-Chairman, Session on Stability, Formulation and Quality Control, and Invited Speaker in the Protein Folding Session, Ninth Internat'l. Biotechnology Symposium and Exhibition, 1992.

Invited Speaker, MIT Biotechnology Process Engineering. Center Workshop on "Proteins as Pharmaceuticals: Structure, Function and Stability", 1992.

Invited Speaker, Gordon Conference on Drug Carriers in Biology and Medicine, 1992.

Keynote Speaker, Protein Formulation and Delivery, 205th ACS Nat'l. Meeting, 1993.

Invited Speaker, Protein Stability Conference, Breckenridge, CO, 1994.

Section Editor (Biologicals and Immunologicals): *Expert Opinion on Investigational Drugs*, 1993-Present.

Associate Editor (Biotechnology), Journal of Pharmaceutical Sciences, January 1, 1994-Present.

Editorial Board (Journal of Biological Chemistry), January 1, 1998-.

Keynote Speaker, Cell Bioengineering Conference, San Diego, CA, 1994.

Organizer with R. Langer and J. Cleland, 1995 and 1997, Formulation and Drug Delivery Conference, Boston.

NIGMS Biomedical Research and Research Training Review Committee, 1996-1999.

AAPS Roche Biotechnology Award, 1999

VI. Teaching Experience

Classes taught in the following areas:

General Biochemistry (all levels)
Physiological Chemistry
General Chemistry
General Biology
Physical Chemistry
Physical Biochemistry
Clinical Chemistry
Biotechnology
Immunology
Science in 20th Century Literature
Political, Societal and Biological Aspects of Cancer
Membrane Biology
Protein Folding
Vaccines
Gene Therapy

VII. Publications (Abstracts and Patents Not Included)

(1976) Middaugh, C.R. and MacElroy, R.D. The Effect of Temperature on Ribose-5-phosphate Isomerase from a Mesophile, Thiobacillus thioporus, and a Thermophile, Bacillus caldolyticus. J. Biochem., 79, 1331-1344.

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Parameters Inferred from Amino Acid Coomposition. Int. J. Pept. Protein Res., 10, 39-50.

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(1977) Middaugh, C.R., Thomas, Jr., G.J. Prescott, B., Aberlin, M.E. and Litman, G.W. Investigations of the Molecular Basis for the Temperature-Dependent Insolubility of Cryoglobulins. II. Spectroscopic Studies of the IgM Monoclonal Cryoglobulin McE. Biochemistry 16, 2986-2994.

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(1978) Middaugh, C.R., Kehoe, J.M., Prystowsky, M.B., Gerber-Jenson, B., Jenson, J.C. and Litman, G.W. Molecular Basis for the Temperature-Dependent Insolubility of Cryoglobulins. III. Structural Studies of the IgM Monoclonal Cryoglobulin MCE. Immunochem. 15, 171-187.

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(1979) Schneider, A.S., Middaugh, C.R. and Oldewurtel, M.D. Role of Bound Water in Biological Membrane Structure: Fluorescence and Infrared Studies. J. Supramolecular Struct. 10, 265-275.

(1979) Middaugh, C.R., Gerber-Jenson, B., and Litman, G.W. Effect of Chemical Modification on the Cold-Induced Insolubilization of Monoclonal Cryoimmunoglobulins. J. Clin. Lab Immunol. 1, 141-145.

(1979) Middaugh, C.R., Tisel, W., Haire, R. and Rosenberg, A. Determination of the Thermodynamic Activities of Saturated Protein Solutions. J. Biol. Chem. 254, 367-370.

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(1991) Matsuno, K., Lewis, R.V. and Middaugh, C.R. "The Interaction of Gamma-Crystallins with Model Surfaces." Arch. Biochem. Biophys., 291, 349-355.

(1991) Dabora, J.M., Sanyal, G. and Middaugh, C.R. "Effects of Polyanions on the Refolding of Human Acidic Fibroblast Growth Factor." J. Biol. Chem., 266, 23637-23640.

(1992) Mach, H., Middaugh, C.R., and Lewis, R.V. "Statistical Determination of the Average Values of the Extinction Coefficients of Tryptophan and Tyrosine in Native Proteins." Anal. Biochem., 200, 74-80.

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